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厦门大学

硕 士 学 位 论 文

基于内源性大麻素系统探讨皂术茵陈方抗大鼠非酒精性脂肪性肝炎的作用机制

Effect and mechanism of ZaoZhuYinchenhao Decoction against Non-alcoholic steatohepatitis in rats based on the Endogenous Cannabinoid system

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摘 要

目的：

1. 观察皂术茵陈方改善大鼠非酒精性脂肪性肝炎的效果；

2. 从内源性大麻素系统探讨皂术茵陈方抗非酒精性脂肪性肝炎的作用机制。

方法：

实验分两部分进行。

1．皂术茵陈方改善大鼠非酒精性脂肪性肝炎的药效学实验：将 40 只雄性 Wistar 大鼠按照随机数字表法随机分成正常组、模型组、皂术茵陈方组和盐酸吡格列酮组。除正常组外，其它各组大鼠给予高脂饮食喂养 12 周，从第 7 周开始，给予对应的药物持续灌胃 6 周。实验结束后处死大鼠，收集肝脏和血清标本，并记录相应数据(体重、肝湿重)。用相应的生化检测方法检测肝组织匀浆 ALT、AST 活性、TG、T-CHO、HDL、LDL、MDA、SOD 含量。并观察肝组织肉眼及病理组织石蜡切片变化(HE 染色)，进行肝组织 NAS 积分。统计分析所得数据。

2．基于内源性大麻素系统探讨皂术茵陈方改善大鼠非酒精性脂肪性肝炎的作用机制研究：动物造模、分组及标本留取方法同上。采用 LC-MS/MS 方法检测大鼠肝组织中内源性大麻素 AEA、2-AG、PEA、OEA 的含量；采用 Real-time PCR 检测大鼠肝组织中内源性大麻素受体 CB1、CB2 及水解酶 FAAH、MGL 的基因表达水平；采用 Western blot 方法检测大鼠肝组织中内源性大麻素受体 CB1 及水解酶 FAAH、MGL 的蛋白表达水平。统计分析所得数据。

结果：

1．皂术茵陈方改善大鼠非酒精性脂肪性肝炎的药效学实验：与正常组相比，模型组大鼠体重、肝湿重、肝指数、NAS 评分、肝组织 ALT、 AST、TG、T-CHO、LDL、MDA 皆显著升高，SOD 活力显著降低，肉眼观察及石蜡切片显示肝组织存在明显脂肪变性，并出现严重炎症损伤；与模型组相比，皂术茵陈方组和盐酸吡格列酮组大鼠的体重、肝湿重、NAS 评分、肝组织 ALT、 AST、TG、T-CHO、LDL、MDA 皆降低明显，皂术茵陈方组大鼠肝组织 SOD 活力明显升高，肉眼观察及石蜡切片可见肝组织脂肪变性及炎症程度均有不同程度的改善。

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2．基于内源性大麻素系统探讨皂术茵陈方改善大鼠非酒精性脂肪性肝炎的作用机制研究：

LC-MS/MS 结果显示：与正常组相比，模型组大鼠肝组织 2-AG、OEA、PEA 含量明显升高；与模型组相比，皂术茵陈方组大鼠肝组织 2-AG、OEA、PEA 含量明显降低。

Real-time PCR 结果显示：与正常组相比，模型组大鼠肝组织 CB1、CB2 的基因表达水平显著升高，FAAH、MGL 的基因表达水平显著降低；与模型组相比，皂术茵陈方组大鼠肝组织 CB1、CB2 的基因表达水平显著降低，FAAH、MGL 的基因表达水平显著升高。

Western blot 结果显示：与正常组相比，模型组大鼠肝组织 CB1 的蛋白表达水平显著升高，FAAH、MGL 的蛋白表达水平显著降低；与模型组相比，皂术茵陈方组大鼠肝组织 CB1 的蛋白表达水平显著降低，FAAH、MGL 的蛋白表达水平显著升高，此结果与本实验中 Real-time PCR 检测结果相符合。

结论：

1．单纯高脂饮食可以成功造出典型的大鼠非酒精性脂肪性肝炎模型。

2．皂术茵陈方可以降低大鼠肝组织 ALT、AST 活性，具有明显的改善非酒精性脂肪性肝炎大鼠炎症损伤的作用。

3．皂术茵陈方可以明显降低肝组织 TG、T-CHO、LDL 含量，具有明显的改善非酒精性脂肪性肝炎大鼠脂质代谢的作用。

4．皂术茵陈方能明显降低 MDA 含量，升高 SOD 活力，具有明显的改善非酒精性脂肪性肝炎大鼠氧化应激损伤的作用。

5．皂术茵陈方能降低 2-AG、OEA、PEA 含量，降低 CB1、CB2 的基因表达和 CB1 的蛋白表达，升高 FAAH、MGL 的基因和蛋白表达水平。通过调控内源性大麻素系统，可能是其治疗非酒精性脂肪性肝炎的作用机制之一。

关键词：非酒精性脂肪性肝炎 皂术茵陈方 内源性大麻素系统

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Abstract

Objective:

1. Observe the pharmacological effect of using ZaoZhuYinchenhao Decoction to treat rats with non-alcoholic steatohepatitis;

2. To investigate the mechanism of action using ZaoZhuYinchenhao Decoction to improve the rats with non-alcoholic steatohepatitis based on the Endogenous Cannabinoid system.

Method:

The experiment was divided into two parts.

1. Pharmacodynamics of using ZaoZhuYinchenhao Decoction to treat rats with non-alcoholic steatohepatitis:40 male Wistar rats were randomly divided into normal group,model group,treatment group including Pioglitazone hydrochloride group and ZaoZhuYinchenhao Decoction group. Except the normal group, other groups were fed with high-fat diet for 12 weeks, treatment group were given the corresponding drugs for 6 weeks,since the seventh week. After the experiment, all rats were sacrificed and their livers and serum samples were collected.Meanwhile,we recorded their body weight and liver weight.ALT and AST Activity, HDL, LDL, TG, T-CHO, SOD, MDA content were detected by biochemical instrument.Liver tissues' pathological changes were observed with naked eye and light microscope after Hematoxylin-Eosin dyeing. Liver tissues' NAFLD Activity Scores were integrated. Finally, we analyze the data.

2.Investigating the mechanism of using ZaoZhuYinchenhao Decoction to improve the rat model of non-alcoholic steatohepatitis based on the Endogenous Cannabinoids system:Detecting endocannabinoids AEA, 2-AG, PEA, OEA's content in rats liver tissue by LC-MS/MS analysis;Detecting CB1, FAAH and MGL's gene expression level in rats liver tissue by real-time PCR； Detecting CB1,FAAH and MGL's protein expression in rats by Western blot analysis.Finally, we process and analyze these data.

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Results:

1. For Pharmacodynamics of using ZaoZhuYinchenhao Decoction to treat rats with non-alcoholic steatohepatitis:①Compared with normal group, the model group rat's weight,liver weight,liver weight ratios,NAFLD Activity Scores,ALT activity,AST activity,TG,T-CHO,LDL and MDA content increased substantially, while the SOD activity dropped by a large margin. The liver tissues' pathological changes observed with naked eye and light microscope both indicating fatty degeneration and inflammatory injury.Compared with model group,the ZaoZhuYinchenhao Decoction group and Pioglitazone hydrochloride group rat's weight,liver weight,liver weight ratios,NAFLD Activity Scores,ALT activity,AST activity,TG,T-CHO,LDL and MDA content declined substantially,while the SOD activity of ZaoZhuYinchenhao Decoction group rose obviously.The liver tissues' pathological changes observed with naked eye and light microscope were both improved to some extent.

2. For investigating the mechanism of action using ZaoZhuYinchenhao Decoction to improve the rats with non-alcoholic steatohepatitis based on the Endogenous Cannabinoids system:①LC-MS/MS results show:Compared with normal group,the model group rat livers's content of the endocannabinoids 2-AG,OEA and PEA increased apparently;Compared with model group,the ZaoZhuYinchenhao group rat livers's content of the endocannabinoids 2-AG,OEA and PEA decreased significantly.②Real-time PCR results show:Compared with normal group,the model group rat livers's m-RNA expression of CB1 and CB2 lifted apparently,while the m-RNA expression of FAAH and MGL droped significantly;Compared with model group,the ZaoZhuYinchenhao group rat livers's m-RNA expression of CB1 and CB2 droped dramatically,while the m-RNA expression of FAAH and MGL lifted noticeably.③Western blot analysis show:Compared with normal group,the model group rat livers's protein expression of CB1 rose obviously,while the protein expression of FAAH and MGL lowered dramatically;Compared with model group,the ZaoZhuYinchenhao group rat livers's protein expression of CB1 lowered apparently,while the protein expression of FAAH and MGL rose definitely.

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Conclusion:

1.By adopting high-fat diet,we can successfully produce the rat's non-alcoholic steatohepatitis liver disease;

2.ZaoZhuYinchenhao Decoction can effectively reduce rat's liver tissue ALT, AST activity, thus it can have great effect on anti-inflammatory injury of rats with NASH;

3.ZaoZhuYinchenhao Decoction can effectively lessen liver tissue's TG, T-CHO，LDL content.Therefore, it can amend the metabolism of lipids of rats with NASH;

4. ZaoZhuYinchenhao Decoction can effectively decrease liver tissue's MDA volume, and increase SOD activity,therefore, it can improve the oxidative stress of rats with NASH;

5.ZaoZhuYinchenhao Decoction can distinctly decrease liver tissue's endocannabinoids 2-AG,OEA and PEA content，lower the m-RNA expression of CB1 and CB2,meanwhile, recede the protein expression of CB1.Additionally,it can upraise the protein and m-RNA expression of FAAH and MGL.Through the regulation of the Endogenous Cannabinoids system, which may be its mechanism of the treatment with NASH.

Keywords:NASH;ZaoZhuYinchenhao Decoction;Endogenous Cannabinoids system

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英 文 缩 略 词 表

中文名称	英文全名	英文缩写
非酒精性脂肪性肝炎	Non-alcoholic Steatohepatitis	NASH
非酒精性脂肪性肝病	Non-alcoholic Fatty Liver Disease	NAFLD
内源性大麻素系统	Endogenous Cannabinoid System	ECS
内源性大麻素	Endocannabinoids	eCBs
大麻素受体	Cannabinoid Receptor	CBR
胰岛素抵抗	Insulin Resistance	IR
游离脂肪酸	Free Fattty Acid	FFA
NAFLD 活动度积分	NAFLD Activity Scores	NAS
苏木精-伊红染色	Hematoxylin-Eosin	HE
丙氨酸氨基转移酶	Alanine Aminotransferase	ALT
天冬氨酸氨基转移酶	Aspartate Aminotransferase	AST
甘油三酯	Triglyceride	TG
总胆固醇	Total Cholesterol	T-CHO
高密度脂蛋白胆固醇	High Density Lipoprotein-cholesterol	HDL
低密度脂蛋白胆固醇	Low Density Lipoprotein-cholesterol	LDL
极低密度脂蛋白胆固醇	Very Low Density Lipoprotein	VLDL
超氧化物歧化酶	Superoxide Dismutase	SOD
丙二醛	Malonaldehyde	MDA
液相色谱-质谱联用	Liquid chromatography-mass spectometry	LC-MS/MS
N-花生四烯酰乙醇胺	N-Arachidonoylethanolamine	AEA
2-花生四烯酰甘油	2-Arachidonoylglycerol	2-AG
N-十六酰胺乙醇	N-Palmitoylethanolamine	PEA
N-油酰乙醇胺	N-Oleoylethanolamine	OEA
脂肪酸酰胺水解酶	Fatty Acid Amide Hydrolase	FAAH
单酰基甘油脂肪酶	Monoacylglycerol Lipase	MAGL

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引 言

非酒精性脂肪性肝炎(Non-alcoholic Steatohepatitis,NASH)是非酒精性脂肪性肝病(Non-alcoholic fatty liver disease,NAFLD)病程中肝细胞内出现脂肪堆积、炎症损伤坏死的病理阶段。现代临床研究表明，NASH 患者有进展为其相关肝硬化、肝癌的风险。目前，全世界范围 NAFLD 发病率逐年增高，发病年龄逐年下降，已成为严重影响全球人们身体健康的慢性肝脏疾病。

尽管现代科学技术不断发展，现代医学水平不断提高，人们对 NASH 的发病机制至今尚未十分明确。Day 等人提出的“二次打击”理论是目前学术界公认的 NAFLD 的经典发病机制[1]。

NAFLD 的发生与进展主要包括机体出现胰岛素抵抗(Insulin Resistance,IR)、游离脂肪酸(Free Fattty Acid,FFA)肝内堆积、瘦素抵抗、炎症反应、氧化应激损伤等[2-3]。近年来的流行病学调查显示：目前 NAFLD 已成为发达国家第一大慢性肝病，在发达国家受 NAFLD 影响的人群大约占人口总数 20%-30%[4]。NAFLD 患者中大约 10%～20%日后可进展为 NASH，NASH 患者 10 年内肝硬化发生率高达 25%，远远高于普通正常人。随着中国经济水平不断发展，人民群众生活水平不断提高，近年来国内 NAFLD 发病率也不断升高，特别是在经济发达的城市，成为仅次于病毒性肝炎的第二大肝脏常见疾病[5-6]。由于 NAFLD 在疾病前期往往无明显的症状，患者多在中后期才出现临床症状。如果治疗不当，其后可恶化进展为其相关的肝纤维化、肝硬化、肝癌，而且它与肥胖、糖尿病、动脉粥样硬化性心脏病等代谢相关疾病关系密切，因此对该病的临床研究具有非常重要的社会意义和经济学意义[7]。

对于 NAFLD 的治疗，西方现代医学以基础疗法为主，手术治疗为辅。当疾病进展为 NASH 时，以药物对症治疗为主。虽然目前临床上对症治疗 NASH 药品种类繁多，但是目前仍然缺乏足够多的实验及临床证据找到治疗 NASH 的特效药[8]。与现有的西药相比，传统中医药在防治脂肪性肝炎方面具有明显优势，临床研究表明中药在改善血脂含量和血清转氨酶水平具有较好的疗效，且不良反应少[9]。近年来中药治疗 NASH 已经成为国内外的一大科研热点，且已证实某些中药有效成分能够改善肝脏组织学指标，但缺乏明确的作用机制及其作用靶点。

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